REMARKS

Favorable reconsideration, reexamination, and allowance of the present patent application are respectfully requested in view of the foregoing amendments and the following remarks. The foregoing amendments have full support throughout the specification. No new matter is entered.

Amendments

Claim 1 is amended. Claim 20 is cancelled. Claims 7-19 are withdrawn.

Rejections under 35 U.S.C. § 112, 1st paragraph

In the Office Action, beginning at page 3, Claims 1-6 and 20 were rejected under 35 U.S.C. § 112, 1st paragraph, as allegedly failing to comply with the written description requirement. Applicants respectfully request reconsideration of this rejection

Although Applicants do not necessarily agree with the basis for the rejection, the claims have been amended to recite the actions as increasing Fhl2 expression or activity, increasing Fhl2/RunX interaction, or determining the formation of ECM. As the Examiner has expressly stated that these actions are fully described by the specification, the claims clearly contain only subject matter which is completely and adequately described, and the claims clearly convey to one skilled in the art that Applicants were in possession of the invention at the time of filing.

For at least the foregoing reasons, Applicants respectfully submit that the Claims fully comply with the written description requirement of 35 U.S.C. §112, 1st paragraph, and therefore respectfully request withdrawal of the rejection thereof under 35 U.S.C. § 112, 1st paragraph.

In the Office Action, beginning at page 4, Claims 1-6 and 20 were rejected under 35 U.S.C. § 112, 1st paragraph, as allegedly failing to comply with the enablement requirement. Applicants respectfully request reconsideration of this rejection

Although Applicants do not necessarily agree with the Examiner's basis for the rejection, the claims have been amended to recite the actions as increasing Fhl2

expression or activity, increasing Fhl2/RunX interaction, or determining the formation of ECM. As the Examiner has expressly stated that these actions are enabled by the specification, the full scope of the claims is clearly enabled.

For at least the foregoing reasons, Applicants respectfully submit that the Claims fully comply with the enablement of 35 U.S.C. §112, 1st paragraph, and therefore respectfully request withdrawal of the rejection thereof under 35 U.S.C. § 112, 1st paragraph.

Rejection under 35 U.S.C. §112, 2nd paragraph

In the Office Action, beginning on page 5, Claims 1-5 and 9 were rejected under 35 U.S.C. §112, 2nd paragraph, as allegedly being incomplete for allegedly omitting essential structural cooperative relationships of elements. Applicants respectfully request reconsideration of this rejection.

First, claim 9 is not under examination. Although Applicants do not necessarily agree with the basis for this rejection, the claims have been amended to connect the intended outcome with the recited method steps. It is asserted that there are no omissions in the steps of the method, and that the skilled artisan would understand what is encompassed by the method.

For at least the foregoing reasons, Applicants respectfully submit that the Claims fully comply with the enablement of 35 U.S.C. §112, 2nd paragraph, and therefore respectfully request withdrawal of the rejection thereof under 35 U.S.C. § 112, 2nd paragraph.

Rejection under 35 U.S.C. §103(a)

In the Office Action, beginning at page 6, Claims 1-6 and 20 were rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Lai et al. (hereinafter "Lai") in view of Marie *et al.* (hereinafter "Marie"), Amaar *et al.* (hereinafter "Amaar") and Muller *et al.* (hereinafter "Muller"). Applicants respectfully request reconsideration of this rejection.

The claims are drawn to positive *in vivo* steps which correlate the *in vitro* action with an *in vivo* result. Such a correlation has never been shown before. Furthermore,

neither Amaar nor Lai teach or suggest a screening method, but merely recognized the effect of Fhl2 overexpression. In addition, both Amaar and Lai fail to show expression of Fhl2 in osteoblasts *in vivo,* which is an explicit step of the claims. Amaar describes detection of expression of Fhl2 by Northern Blot (page 12056, Figure 3) and Western Blot (page 12057, Figure 4) in permanent cell lines. These cell lines are either permanent cell lines which were obtained from osteosarcomas of human origin (U-2 OS, MG-63, Saos) or primary cell lines which stem from the calvaria and the ribs and have been passaged three to four times (page 12054, right-hand column, third paragraph). Amaar did not analyze if Fhl2 is expressed in osteoblasts. Contrary thereto, the inventors show that expression of Fhl2 *in vivo* can be detected in osteoblasts which are already differentiated in the bone of mice.

Lai hypothesize that Fhl2 may play an important role in bone formation but they have not been able to prove their speculation to this day. This is very well corroborated by the fact that the same group after four years of additional analysis is not able to link any function of Fhl2 to bone formation in untreated mice (See Cheng et al., 2006, submitted with the response filed July 23, 2009, and discussed in more detail herein).

Although the claims are directed to the identification of a compound which can form an extracellular matrix in osteoblasts *in vivo*, it was clearly unknown in the prior art whether the *in vitro* effects can really be applied to an *in vivo* situation. Applicants have clearly shown that Fhl2 has an effect both *in vitro* and *in vivo*, and such a correlation is an explicit positive step in the claims. Furthermore, the disclosures of these two references cannot render the claimed invention obvious because the detection *in vivo* verus *in vitro* is not just a mere technical difference. The inventors have shown that Fhl2 expression can be induced in all tissues examined by the inventors to date, as soon as the cells are taken into culture, irrespective of whether or not the tissue already expresses Fhl2 *in vivo*. Furthermore, Amaar only mention expression in human osteoblasts, but not *in vivo* expression of Fhl2 in osteoblasts. Thus, induction of Fhl2 expression in cell culture cells is an indirect and unspecific effect which does not allow any conclusion with regard to the role in bone formation.

Lai do not show that Fhl2 enhanced osteoblast growth and differentiation, as argued in the Office Action; however, Lai do show that Fhl2 regulates MC3T3-E1

proliferation and differentiation. MC3T3-E1 cells do not in any way resemble osteoblasts, but are uncommitted precursors with the potential to differentiate upon stimulation.

Amaar present the hypothesis that IGFBP-5 may bind to Fhl2, a transcription modulator, to stimulate transcription of putative IGFBP-5 target genes that may be involved in regulation of osteoblast cell proliferation and differentiation (page 12059, right-hand column, last sentence of second paragraph). While this hypothesis is regarded as speculative by the authors themselves, it clearly refers to a role of Fhl2 on proliferation and differentiation of osteoblast precursors. It is therefore perfectly possible that there is a general effect of Fhl2 on the cell division rate and on the differentiation process as it has been described for Fhl2 in multiple cell culture systems from varying tissues. This general *in vitro* effect of Fhl2 would inevitably have some effect on osteoblast precursors in the form of an indirect and unspecific effect on the mineralization and expression of osteocalcin after these cells have differentiated into mature osteoblasts.

The increase observed in proliferation and differentiation in cell culture observed by Lai after overexpression of Fhl2 is most likely an unspecific general effect found in many different cell culture systems using cells from various origins. Accordingly, one of ordinary skill in the art would not have expected that the effects observed by Lai to be specific or applicable to bone cells. Other groups have been unable to demonstrate these effects *in vivo*, which is supported by the finding that Fhl2 has no effect on proliferation or differentiation of osteoblasts *in vivo*. Furthermore, the unspecific effect in cell culture observed by Lai may also indirectly influence mineralization and expression of osteocalcin.

Marie is cited for the teaching that FGF2 is an essential factor involved in skeletal development, bone formation, and that it regulates the proliferation of osteoblasts, but none of their reports regarding FGF2 are relevant or even mentioned in the context of Fhl2. Muller is cited for teaching the Fhl2 is a transcriptional co-activator and that it binds and selectively activates the transcriptional activity of androgen receptors, as well as teaching synthetic compounds that are capable of activating the signal pathway that stimulates Fhl2 expression in cells. It is asserted that none of these teachings, including Amaar, cure the deficiencies of the teachings of Lai, and therefore, none of the teachings

of the references, either singly or in combination, render the claimed invention obvious. The teachings are entirely disparate, and one of ordinary skill in the art would not have had a reason or motivation to combine these teachings, and even so, would clearly not have arrived at the claimed method.

Contrary to these teachings, the inventors of the present application have shown a direct specific cell-autonomous and anabolic effect of Fhl2 on the activity of already differentiated osteoblasts *in vitro* and *in vivo*. In addition, the present inventors are able to present a molecular mechanism for this observed effect. It is also noteworthy that the present inventors could not detect any Fhl2 effect on proliferation of differentiation of osteoblasts or osteoblast precursors *in vivo* which contradicts the *in vitro* data of Amaar.

Specifically regarding the rejection over Lai, the group of Su-Li Cheng investigated the function of the co-factor FHL2 in the formation and maintenance of bone. The Lai reference cited by the Examiner is an abstract published in 2002 and only reports preliminary data of this work. The follow-up paper of this investigation was published in 2006, that is, ***after the filing date of the subject application***. A copy of this later publication by the Cheng group was submitted with the response filed July 23, 2009.

The Lai abstract describes that FHL2 interacts with avβ5 Integrin. FHL2 localizes intracellularly at the focal adhesions and in the nucleus. Ectopic expression of FHL2 in the MC3T3-E1 cell line which exhibits characteristics of osteoblast precursors, led to increased cell adhesion, matrix mineralization and cell proliferation. There is no reliable information, however, on the reason for these effects. Based on the data of the Lai abstract, an accelerated differentiation of osteoblast precursors and/or the enhanced proliferation would be the most obvious speculation. The preliminary observations are summarized at the end:

"FHL2 upregulates osteablast growth and differentiation and synergizes Cbfal and FGF2 activity. Thus, FHL2 may play an important role in bone formation."

In the later full publication, Lai could not confirm any of the postulated functions of FHL2 *in vivo* with untreated wild-type and knockout mice. Again they report that FHL2 stimulates osteoblast differentiation and proliferation.

The inventors of the subject application observed that female as well as male FHL2 deficient mice with a congenic C57BL/6 background showed a significant and remarkable loss in bone substance of about 30%. They could demonstrate that the observed osteopenia was alone due to a reduced osteoblast activity and not due to a differentiation of osteoblast precursors and also not due to a change in cell proliferation. This finding was confirmed in the inventor's later publication Gunther et al. (copy submitted with the response filed July 23, 2009). Accordingly, the inventors used for verification a cell line of already differentiated osteoblasts (7F2). In addition, investigation of *ex vivo* cultures of primary osteoblasts from wild type and FHL2 deficient mice confirmed their analysis that FHL2 *in vivo*, *ex vivo,* and in 7F2 cells had only an anabolic effect on osteoblasts but not on their differentiation. This analysis is corroborated by the fact that the ectopic expression of FHL2 in osteoblasts in transgenic mice leads to an increase in bone cells due to enhanced anabolic activity. However, the inventors did not observe any change in osteoblast differentiation or proliferation in any of the systems investigated.

Lai do mention an increased osteocalcin expression as a consequence of ectopic FHL2 expression. The observed change in osteocalcin expression, however, on the basis of the data of Lai, could also be an indirect effect which has nothing to do with FHL2. Contrary to the inventors' work, no functional relationship is described by Lai et al., let alone a molecular mechanism. The present inventors could demonstrate that FHL2 and RUNX2 form a complex on chromatinized osteocalcin promoter *in vivo* and that FHL2 acts as co-activator in dependence from RUNX2.

Remarkably, the group of Cheng could also four years after publication of the Lai et al. abstract not show any bone phenotype in FHL2 deficient mice (see Cheng). The analysis of the bone substance was made only with μCT. A detailed investigation in not decalcified bone slices was not conducted. Maybe these different observations were made because Lai did not use congenic mice. However, a uniform genetic background (congenic mice) is crucial for investigations of the bone substance, as the bone substance varies up to more than 30% among different in-bred mouse strains.

In summary, despite their investigation of FHL2 deficient mice, *ex vivo* cultures from calvaria and ectopic expression of FHL2 in MC3T3-E1 cells, Lai at no time described an anabolic function of FHL2 in osteoblasts.

Therefore, the primary reference of Lai has been effectively argued, and taken in conjunction with the presented evidence, which was submitted in response to the last Office Action but was not addressed by the Examiner, must be removed as effective prior art, either alone or in combination with any other reference. For the reasons presented above, there is no reason or motivation to combine the teachings of Lai with any other reference and arrive at the claimed invention.

For at least the foregoing reasons, Applicants respectfully submit that the subject matters of the Claims, each taken as a whole, would not have been obvious to one of ordinary skill in the art at the time of Applicant's invention, are therefore not unpatentable under 35 U.S.C. § 103(a), and therefore respectfully request withdrawal of the rejection thereof under 35 U.S.C. § 103(a).

Conclusion

For at least the foregoing reasons, Applicants respectfully submit that the present patent application is in condition for allowance. An early indication of the allowability of the present patent application is therefore respectfully solicited.

If Examiner Hiriyanna believes that a telephone conference with the undersigned would expedite passage of the present patent application to issue, he is invited to call on the number below.

It is not believed that extensions of time are required, beyond those that may otherwise be provided for in accompanying documents. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and the Commissioner is hereby authorized to charge fees necessitated by this paper, and to credit all refunds and overpayments, to our Deposit Account 50-2821.

Respectfully submitted,

By: /Shelly Guest Cermak/
Shelly Guest Cermak
Registration No. 39,571

U.S. P.T.O. Customer No. 36844
Cermak Kenealy Vaidya & Nakajima LLP
515 E. Braddock Road
Alexandria, VA 22314
703.778.6608

Date: April 26, 2010